JOURNAL OF BACTERIOLOGY, Jan. 2007, p. 298–304 Vol. 189, No. 2
0021-9193/07/$08.00+0 doi:10.1128/JB.01215-06

MINIREVIEW

A New Look at Bacteriophage λ Genetic Networks[▽]

Donald L. Court,[1]* Amos B. Oppenheim,[1,2,3] and Sankar L. Adhya[3]

Gene Regulation and Chromosome Biology Laboratory, National Cancer Institute at Frederick, Frederick, Maryland[1]; Department of Molecular Genetics and Biotechnology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel[2]; and Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland[3]

Bacteriophage λ is a temperate bacteriophage, meaning that it can reproduce and develop either in a lytic or lysogenic state. When λ infects its bacterial host *Escherichia coli*, the phage may develop lytically, causing cell lysis with the release of hundreds of progeny virus, or it may abort lytic development by switching off most viral expression, integrate its genome into the bacterial chromosome, and exist as a quiescent prophage in the lysogenic state. Although very stable, the lysogenic or prophage state can be reverted by inducing agents that damage the host DNA, returning the virus λ to its lytic state. These systems of lytic growth, lysogenic growth, and lysogenic induction from the prophage state are excellent model systems for understanding developmental pathways and the switches between these pathways (29, 45). Within these pathways are sets of intertwined positive and negative regulators of gene expression acting at the transcription and posttranscription level, which have been studied extensively for more than 40 years. These λ paradigms of developmental pathways and regulatory functions, although well established, continue to evolve with surprising discoveries and accumulated insights.

There is a great interest in using systems biology approaches and system theory to understand all interactive processes in a cell and in an organism. This systems approach depends upon generating enormous amounts of data that describe all gene sequences and their transcript and protein levels, as well as their regulatory controls and dynamic interactions. Mathematical models based on this information should then be able to explain the various networks and to make predictions concerning any perturbation of the system. However, models are only as good as the data used to generate them, and good models also depend upon their testability in different genetic and environmental conditions. This will be an intimidating job for most complex organisms as we can infer by the various attempts to describe the genetic and developmental networks of a simple phage like λ (1, 3, 41, 69). Although λ may be the most completely understood organism, we know that there is a lot more to learn (16, 45). This review describes several new findings about λ regulation, which add to previously unknown levels of regulation and question certain dogma and which will be essential for meaningful advances in systems biology.

* Corresponding author. Mailing address: Gene Regulation and Chromosome Biology Laboratory, National Cancer Institute at Frederick, Bldg. 539, P.O. Box B, Frederick, MD 21702. Phone: (301) 846-5940. Fax: (301) 846-6988. E-mail: court@ncifcrf.gov.

▽ Published ahead of print on 3 November 2006.

Lytic development. The bacterial RNA polymerase (Pol) binds and transcribes from the early promoters *pL* and *pR* (Fig. 1). RNA Pol transcribes as far as the transcription terminators *tL1* and *tR1* beyond *pL* and *pR*, respectively (54, 56, 57). The product of the *N* gene, located on the *tL1*-terminated transcript, modifies subsequent RNA Pol molecules initiating at *pL* and *pR* so that they transcribe through not only *tL1* and *tR1* but also other terminators downstream (7, 8; for see reviews, see references 11, 22, 23, and 53). Thus, through a mechanism of transcription antitermination, N allows other genes distal to the *tL* and *tR* terminators to be transcribed. These genes encode various functions of the phage required for either lytic or lysogenic development. The genes in the *pL* operon include those involved in general and site-specific recombination. The genes in the *pR* operon include replication genes *O*, *P*, and *ren*, as well as another positive regulatory gene *Q*. Like N, Q protein is an antitermination factor (55). Q modifies RNA Pol as it initiates at the strong constitutive promoter *pR′* that is the promoter for genes that encode lysis and morphogenetic proteins of the phage. Transcripts from *pR′* normally terminate at *tR′* some 200 bases away (Fig. 1). Q action on RNA Pol prevents *tR′* termination, allowing expression of the rest of the lytic genes beyond Q. Once Q activates these genes, the cell is committed to lysis. Thus, the lytic pathway, activated by N and Q, is a temporal cascade of transcription from the early promoter *pR* to the late promoter *pR′*, providing lytic gene expression through sets of termination and antitermination events.

Lysogenic development. Two phage proteins, Int and CI, are required to form stable lysogens. Int allows the integration of the phage genome into the bacterial chromosome, and CI represses the two early phage promoters to prevent any lytic phage gene expression. When λ first infects, Int and CI are not initially made, and the phage initiates gene expression along a set of events that are common to both the lytic and lysogenic pathways. If conditions are favorable during this initial phase, Int and CI synthesis can be switched on to enable lysogenic development. This activation depends primarily upon the phage CII function.

The *cII* gene is located between the *tR1* terminator and the replication genes and, thus, is transcribed with the early lytic genes. However, CII protein is required only for lysogenic development of infecting phages. Another gene required for lysogenic development is *cIII*, located beyond *tL1* in the *pL* operon. Mutations in these genes as well as in the *cI* gene encoding the repressor function cause λ plaques to be clear,

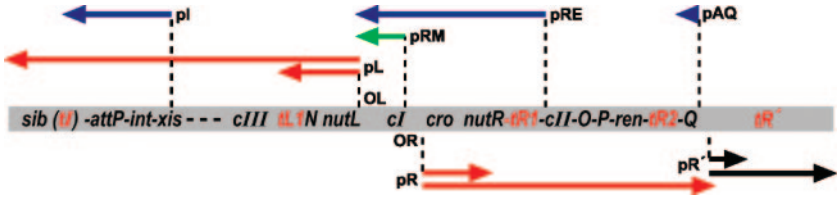

FIG. 1. Gene and transcription map of λ. Genes are shown in the shaded rectangle. The early transcripts for *pL* and *pR* promoters are shown as red arrows. The late transcript from *pR′* is indicated with black arrows. The CII-activated *pI*, *pRE*, and *pAQ* transcripts are indicated with blue arrows. The pRM transcript activated by CI is a green arrow. Transcription terminators (t) are shown as red letters among the genes. The *tI* terminator is indicated in parenthesis because it is contained within the larger *sib* processing site. The operators *OL* and *OR* where CI and *Cro* bind are shown next to the *pL* and *pR* promoters.

unlike the normal turbid plaques where the turbidity indicates growth of lysogenic cells. Whereas the CI repressor is required to maintain the repressed lysogenic state, the CII and CIII proteins are only required to initially activate CI synthesis (34). Once CI has been made, the CII and CIII functions are no longer required because CI can maintain its own synthesis.

Repressor establishment. The CII protein is the primary switch element for establishing repression after infection. CII activates and thereby coordinates transcription from three promoters, which are silent until sufficient CII accumulates. Promoter *pI* transcribes the *int* gene (59), promoter *pRE* transcribes the *cI* gene (60), and promoter *pAQ* transcribes an antisense RNA within the *Q* gene (31) (Fig. 1). Whereas Int and CI proteins are required to form stable lysogens, the Q antisense RNA inhibits Q function and in so doing preempts lethal lytic expression as cells become lysogenic (36).

Coordination in turning on all three of these promoters is required to allow a stable λ lysogenic response with CII acting as the "switchman." Without activation of this switch by CII, λ will follow the lytic pathway, a default course. The tripartite action of CII requires a high threshold level of the protein. The levels of CII are reduced by the bacterial HflB (FtsH) protease, which binds to the C-terminal part of CII, causing its rapid decay (13, 35). Like CII, the CIII protein is also important to establish lysogeny, but unlike CII, its role is indirect. CIII inhibits the bacterial protease HflB (4, 32). Thus, without CIII, HflB degrades CII and not enough CII is available for the switch. Inhibition of HflB by CIII allows accumulation of CII to the threshold level critical for its coordinated action on its three promoters. It is known that a cell infected by one phage predominantly follows the lytic default pathway (about 99% of the time), but infection by more than one phage greatly enhances the lysogenic switch (about 50% of the time). Since calculations indicate that a cell infected by two phage is sufficient to tip the balance in favor of lysogeny (9, 36, 37), it is reasonable to expect that more CIII and CII would be made from twice the number of infecting phage, thus enabling the CII-mediated switch. CIII concentration is important for this higher lysogenic response because of the limiting levels of HflB in cells (65). We will discuss in more detail the regulation of CII expression and its activation of the three silent promoters.

Repressor maintenance. Once CI repressor is made from *pRE*, the repressor shuts off the early promoters *pL* and *pR* by binding at operators *OL* and *OR* (Fig. 1 and 2). This shuts off all λ functions in the *pL* and *pR* operons including CII and CIII, thereby precluding continued CI expression from the CII-activated pRE promoter. CI repressor continues to be made, however, by the enhancement of a weak promoter, *pRM*, located downstream of *pRE*. CI itself activates the *pRM* promoter. Thus, CI acts both as a repressor and an activator.

The *OR* operator is composed of three repressor binding sites, *OR1*, *OR2*, and *OR3*. Three similar sites are present at *OL* (Fig. 2). CI binds tightly and cooperatively to *OR1* and *OR2* as a tetramer to repress *pR*. A similar tetramer bound at *OL* also represses *pL* (48). These two sets of tetramers interact to form an octamer looping the DNA between *OL* and *OR* (Fig. 2) and generating a more stable repression complex of the early promoters (15, 17, 52, 64). In this octamer complex, the CI bound at *OR2* represses *pR* and at the same time activates the adjacent *pRM* promoter to stimulate *cI* transcription. As CI expression increases, the higher CI concentration enables a CI dimer to bind to each of the weaker *OR3* and *OL3* operators in the looped complex (Fig. 2). These dimers inter-

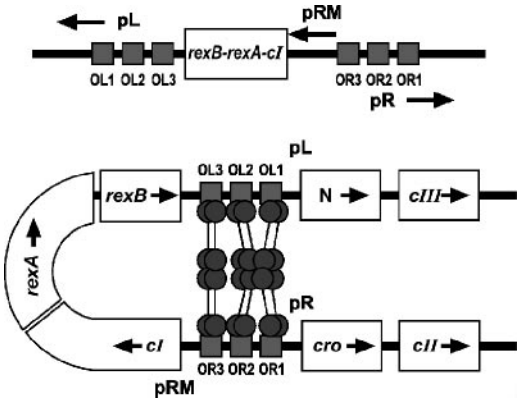

FIG. 2. Looping of the λ operators *OL* and *OR* during CI binding. The promoters *pL* and *pR* control the transcription of the early genes N and cIII and the genes *cro* and cII, respectively (Fig. 1). The promoter *pRM* transcribes *CI*, *rexA*, and *rexB* in a lysogen. The function of the *rex* genes in a lysogen is to exclude certain other phages infecting the cell. In the lower part of the figure, the repressor is shown binding to *OL* and *OR* regions to create a stable looped complex. At each individual operator, e.g., *OL1*, a dimer of repressor forms as indicated by the two dumbbells bound there. A similar dumbbell bound at *OL2* forms a tetramer with the one at *OL1*, and together this tetramer interacts with a second tetramer at *OR1-OR2* to form a looped octamer to tightly repress *pL* and *pR*. A repressor dimer is shown bound at *OR3* interacting as a tetramer with another dimer at *OL3*. This *OR3-OL3* tetramer represses *pRM* to down-regulate CI synthesis. As repressor levels drop in cells, *OL3* and *OR3* become free of repressor, and the *pRM* promoter is activated by repressor bound at *OR2* (not shown).

act with each other and repress CI synthesis by occluding *pRM* (17, 64). This positive and negative autoregulation at *pRM* by CI ensures that a narrow range of CI repressor level is maintained, which is optimum for stable lysogeny but is adjusted low enough for efficient induction of the lysogen. The stable lysogen makes sufficient repressor to block not only prophage lytic development but also that of any extraneous infecting λ phage, thus imparting immunity to the lysogen against lytic superinfection.

Lysogenic induction: the function of CI and Cro. The *cro* gene, the first gene expressed from the *pR* operon, is required for lytic development. Cro is a weak repressor that binds the same *OL* and *OR* sites as CI but with different relative affinities. Cro binds best to *OR3*, by which it blocks CI synthesis from *pRM* (33). These two proteins, CI and Cro, which block each other's transcription, have been described as the elements responsible for the classical bistable λ genetic switch (47). CI is responsible for the immune state by turning off *pL*, *pR*, and Cro. Cro, once made, is able to maintain an anti-immune state by turning off *pRM* and CI expression while leaving *pL* and *pR* and its own expression at least partially on (19, 25, 64). This anti-immune condition, however, can only be established with a genetically modified, defective prophage (19).

Cro has been believed to play an active role in switching lysogenic cells to the lytic state following induction. However, recent findings have raised questions about this role. Originally, a specific *OR3* mutant was shown to prevent Cro repression of pRM and to inhibit prophage induction. From this result, it was proposed that the role of Cro was to aid the transition from lysogeny to lytic development by reducing repressor levels as induction ensued. This is the basis for the classical Cro/CI bistable, genetic switch (47). Yet recent experiments demonstrated that the *OR3* mutation used in the early studies had a second effect. The mutation also prevented CI binding and negative autoregulation, thereby causing higher than normal steady-state levels of CI repressor in the lysogen. This higher level of CI was a cause for the reduced induction (15, 17) reported by Ptashne and his colleagues.

The initial stages of lysogenic induction might be expected to reduce CI functional levels and initiate Cro expression. A recent study in which CI was partially inactivated did not reveal any difference in induction levels in the presence or absence of *cro*, supporting the idea that the CI/Cro bistable switch is not essential for the induction process (64). Atsumi and Little constructed a λ hybrid phage in which the *cro* gene is replaced with the *lacI* repressor gene; in this hybrid, LacI at the *lac* operator controls *pR* transcription during lytic growth (2, 49). In this arrangement, the λ *OR3* operator and *pRM* promoter are unchanged and controlled only by CI repressor; Cro is not present. The hybrid phage can be induced and completes the lytic cycle in the absence of Cro binding to *OR3*, supporting the idea that Cro is not absolutely required for induction. However, Atsumi and Little did notice that, compared to wild-type λ, induction required about a 40% greater level of UV irradiation in the absence of Cro, suggesting that the presence of Cro may aid the induction process at suboptimal UV doses.

The role of Cro in the lytic pathway following infection. It is known that Cro turns down transcription from *pL* and *pR* by four- and twofold, respectively (reference 64 and references therein), thereby reducing CII levels indirectly by its effect on CIII as well as directly by reducing *cII* gene expression (51). By this cumulative reduction of CII levels, Cro helps the default lytic course. Consistent with this, a mutation in *cro* causes an infecting phage to abort the lytic pathway, which can be restored by a second mutation in *cII* (20, 36). This suggests that too much CII protein accumulates in a *cro* mutant.

Regulation of Q activity. There is an unexpected delay period in lytic development between the time of activation of λ genes by N antitermination and the time of activation of the late λ genes from *pR′* by Q antitermination. Although the molecular mechanism is unknown, the delay is believed to be caused by a high threshold requirement for Q in its role of antiterminator, as indicated by direct measurements of Q protein accumulation and Q antitermination activity (36). The delay in committing to the lytic state is likely very important for the ability of λ to switch over to the lysogenic state. This allows time for CII function to be made and for the accumulation of CI and the Q antisense RNA, with the latter blocking Q function. Premature expression of the *Q* gene prevents lysogeny, as evidenced by the effect of a mutation, *byp*, which results in a new promoter just upstream of Q. This promoter causes Q to be expressed immediately after infection, resulting in clear plaque formation (8, 63). Mutation of the *pAQ* promoter prevents the antisense RNA synthesis and also causes clear plaque formation (30, 31) because Q function and lytic expression are not inhibited. How the antisense transcript inhibits Q function remains to be determined. It is not known whether *pAQ* RNA inhibits Q synthesis by an antisense mechanism or Q activity by binding to the protein.

The N-antitermination complex. N is a positive regulator for the lytic pathway. N protein, once made, binds to specific nascent RNA sites NUTL and NUTR. The NUTL site is between the N gene and the *pL* promoter, and NUTR is downstream of the first gene, *cro*, in the *pR* operon (Fig. 1). A set of bacterial proteins called Nus, which takes part in cellular transcriptional and translational processes, interacts with N and RNA Pol during N-mediated transcription antitermination (10, 12, 40, 61). BoxA and BoxB are elements of the RNA Nut sites (24). BoxB is a stem-loop structure in the RNA and binds N (6). BoxB-bound N associates with NusA and RNA Pol. BoxA RNA binds the NusB and NusE complex (43, 46). Thus, N, Nus factors, and Nut RNA interact with RNA Pol while tethered by the nascent RNA transcript (44, 66–68) (Fig. 3). N is the driving force for antitermination, while Nut and the Nus factors provide stability and full activity to the system (14, 50). Although N overrides both Rho-dependent and -independent terminators, the exact way in which N binds and changes the elongating RNA Pol is still being debated (21).

Regulation of N expression. The importance of N′s role as an early regulatory protein of phage λ is reflected by the variety of its own regulation. First, *N* transcription is down-regulated from *OLpL* by CI- and Cro-mediated repression. Second, N is degraded by protease(s), causing a relatively short half-life. Third, and most recently discovered, is that N represses its own translation in conjunction with the bacterial endoribonuclease RNase III, which regulates this autorepression. This rare ability of N to modulate both transcription and translation is described below.

N is the first gene in the *pL* operon (Fig. 1). The 220-nucleotide RNA segment upstream of *N* contains the NutL site

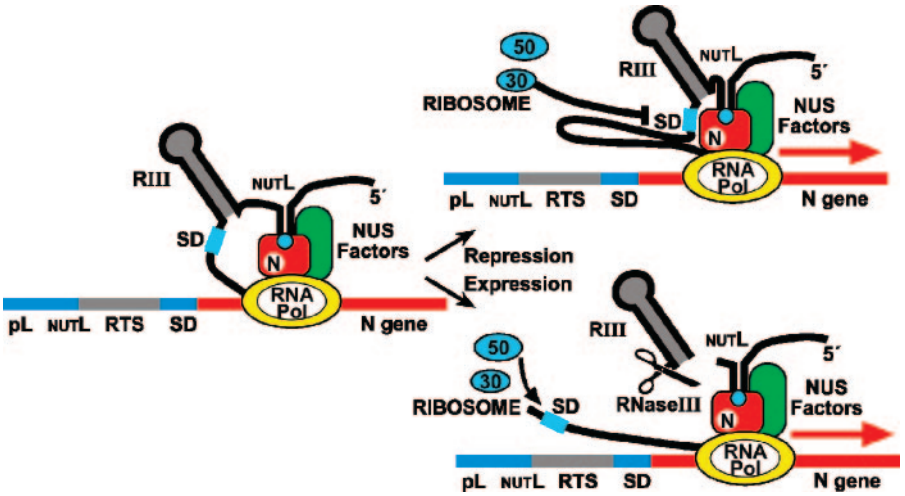

FIG. 3. RNase III control of N-mediated translation repression. RNA Pol is modified by N and Nus factors bound to the NutL RNA structure to become transcription termination resistant. This transcription antitermination complex represses N gene translation (top). If sufficient RNase III endonuclease is present, RNase III cleaves at RIII and dissociates the RNA Pol complex from the N gene-containing transcript, thereby preventing N translation repression. RTS denotes the DNA encoding the RIII RNase III structure, and SD is the N Shine-Dalgarno sequence.

to which N binds, a large stem-and-loop structure following NutL, which is cleaved by RNase III (39, 62), and finally the ribosome binding site (Shine-Dalgarno) of N (Fig. 3). N and RNase III act at their respective binding sites to repress and enhance N translation, respectively (67, 68). When RNA Pol first transcribes the *pL* operon after phage infection, it transcribes through the N gene and terminates at *tL1* (Fig. 1). From these terminated transcripts, the N protein is made. As N accumulates, it binds subsequently initiated *pL* transcripts at NutL and modifies RNA Pol to the N-antitermination form with the help of Nus factors. The N-modified RNA Pol transcribes through *N* and *tL1* as well as distal genes and terminators. N translation from the *pL*-antiterminated transcripts is dependent upon the level of RNase III in cells. If it is high, processing of the RNase III site (Fig. 3) between NutL and the N message is rapid, allowing continuous translation of N from the message. If the RNase III level is low, processing of RIII occurs too slowly, resulting in autorepression of N translation. Since the cellular RNase III level increases and decreases with growth rate (5), N translation also increases and decreases with growth rate (67).

Mechanism of translation repression of N. *N* translation repression is abolished by mutating components of the N antitermination complex such as N, NutL, and Nus factors, or by replacing *E. coli* RNA Pol with T7 RNA Pol for the transcription of *N* (68). Thus, N translation repression is dependent on forming the *E. coli* RNA Pol transcription antitermination complex. The N-antitermination complex, while extending RNA beyond *tL1*, represses *N* gene translation from the same RNA molecule. In contrast, if the N-antitermination complex does not form, the N message is terminated at *tL1*, and the *N* gene is translated. RNase III interferes with translation repression by the N-antitermination complex by splitting the NutL site where the complex is bound from the N message. Ultimately, the level of the global regulator, RNase III, controls *N* gene expression; growth in rich medium leads to high RNase III and N levels, favoring lytic development, whereas growth in poor medium or under starvation conditions leads to low RNase III and N levels, favoring lysogenic development (37, 47).

We previously mentioned the lytic/lysogenic decision in terms of the importance of CII and the multiplicity of infection in causing a switch from lytic to lysogenic growth. High multiplicities of infection ensure that CII accumulates to a threshold allowing the lysogenic switch. This CII multiplicity-dependent switch is epistatic to the N effect in rich or minimal medium (18). On the other hand, infection of a cell by a single phage leads predominantly to lytic development under all growth conditions. However, the number of rare lysogens that occur is dictated to some extent by the growth condition and its effect on N expression. As discussed above, during an infection, λ senses cell growth conditions through RNase III levels, and it is RNase III that affects N levels and lysogeny. When a single phage is infecting a cell in minimal medium where N levels are low, lysogeny increases compared with rich medium infections where N levels are high (37). Is the occurrence of the extremely rare lysogens found during single infections in rich medium stochastic or determined by cellular variables? Since the population of cells being infected is in various stages of the cell cycle, it is difficult to address the issue without infecting synchronous cells.

Retroregulation of *int* gene expression during phage infection. CII is required for Int expression following λ infection. As previously described, *int* gene transcription is activated from the *pI* promoter by CII, and this ensures coordinate synthesis of Int and CI proteins to form a stable lysogen. The finding that CII is required for Int synthesis raised the issue as to why Int is not expressed from the *pL* promoter by N antitermination, even though the N-antitermination complex transcribes the *int* gene. It was demonstrated by genetic studies that a *s*ite *i*n the *b* (*sib*) segment of λ beyond *int* and *attP* (Fig. 1) inhibits Int expression from *pL* but not from *pI* (27). The sequence of the *sib* site revealed the presence of a large stem-loop structure in the RNA, which is sensitive to processing by RNase III (Fig. 4).

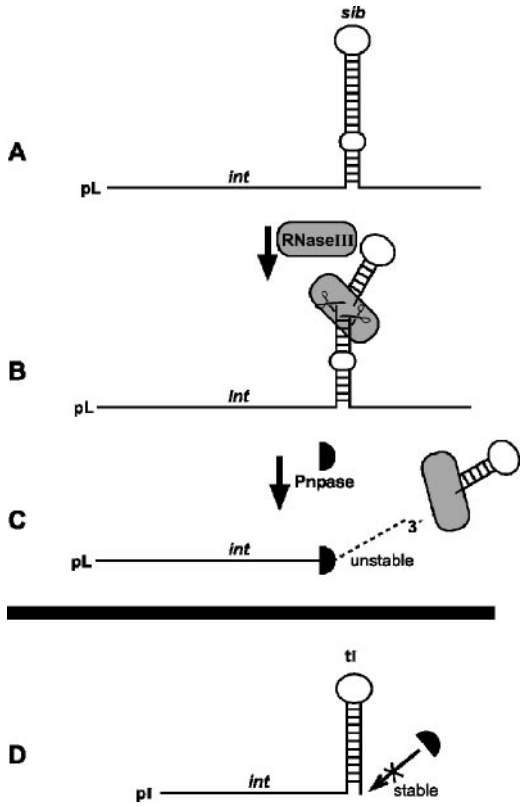

FIG. 4. Regulation of *int* gene expression. (A) Int transcription from *pL* is antiterminated by N and reads through a terminator tI (see panel D) generating an extended stem structure (*sib*). (B) This structure is processed by RNase III to generate a new RNA 3′ end of *int* transcripts that is sensitive to the exonuclease PNPase, shown in panel C. (D) Transcription from *pI* is not antiterminated by N and stops at *tI* to generate a stem that is not processed by RNase III and that is resistant to PNPase.

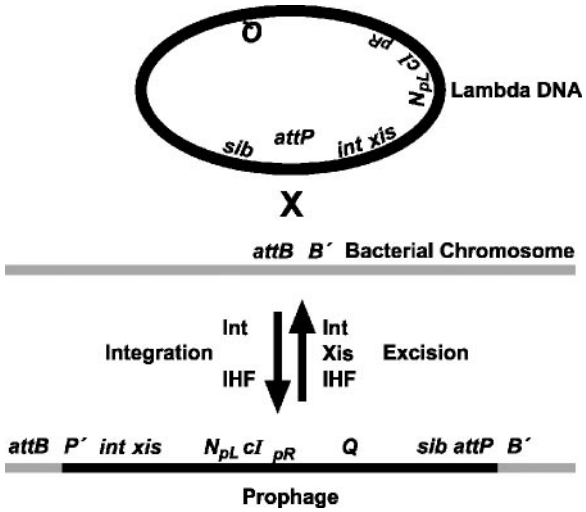

FIG. 5. l DNA integration and excision. The l DNA molecule circularizes following infection (top), and with Int protein of the phage and IHF protein of *E. coli*, recombines at specific sites *attP-P′* in the phage and *attB-B′* in the bacterial chromosome (shown as a linear shaded bar) to integrate. The integration event generates hybrid *att* sites *attB-P′* and *attP-B′* at the prophage DNA (black bar) ends. Following prophage induction, excision occurs between *attB-P′* and *attP-B′* by site-specific recombination. For this recombination the Xis protein of the phage is required in addition to Int and IHF. An important rearrangement of the phage DNA is caused by the integration step. The *sib* site is adjacent to *int* during the phage infection (top). However, integration completely separates *sib* from *int* (bottom) in the prophage. Thus, integration changes the regulation of Int expression from *pL* transcripts.

When λ infects host mutants defective in RNase III, Int is expressed from *pL* transcripts. Thus, processing of the *pL* transcript at Sib by RNase III prevents Int expression. This cleavage event initiates degradation of the *int* transcript from the 3′ processed end by polynucleotide phosphorylase (PNPase). Thus, RNase III and PNPase are both required to retroregulate Int expression from a downstream site (26).

If RNase III processes the *pL* transcript at Sib to prevent Int expression, how does the *pI* transcript avoid being processed? This is because the RNA Pol initiating from *pI* terminates at *tI* without transcribing the entire Sib site, and the transcript is thus resistant to RNase III and PNPase. On the other hand, N-modified transcription from *pL* is resistant to termination at *tI* and extends beyond it, generating the fully sensitive RNase III site, Sib (Fig. 4).

Int expression during prophage induction. Although Int is needed for phage DNA integration after infection, Int along with Xis is required for prophage DNA excision after induction (42). After integration of the phage DNA into the bacterial chromosome via *attP*, the *sib* site is physically separated from *int* and is located on the opposite side of the prophage (Fig. 5). Because the integration process splits the *sib* site from *int*, the prophage *int* gene transcript from *pL* no longer carries Sib, and the RNA is stable. This stable *pL-int* transcript expresses Int and the upstream Xis protein at high levels, allowing the excision to occur immediately following induction (28, 58).

Epilogue. λ was discovered by Ester M. Lederberg in 1951 as a prophage in *E. coli* K12 (38). Our knowledge about λ biology has continued to grow and evolve over the intervening 55 years. During this entire time, λ has served as a paradigm for studying gene regulation and development. Models to explain λ's regulatory and developmental switches have also been evolving as new discoveries continue to be made, right up to the present. The study of λ continues to be important for many reasons, not the least of which is as a paradigm for systems biology studies.

Although λ may be the most completely understood organism, from a systems biology viewpoint there is a lot more to learn (3, 41, 69). In this review, we discuss recent findings that reveal new aspects of λ genetic circuitry that (i) deemphasize the role of the classical Cro/CI bistable switch in lysogenic induction, (ii) show the existence of a DNA operator-repressor loop, and (iii) explain the extreme stability of λ lysogenic immunity. Furthermore, we describe how stability of critical regulatory proteins, like N, CII, and Q, determines their threshold requirements in the temporal context of λ's development and provides a paradigm for all genetic and developmental regulation. It is clear that any systems biology approach to understand the big picture is doomed to fail unless the details of the system are known. Complete and accurate experimental results are crucial for mathematical modeling. Earlier mathematical models of the systems of λ require reevaluation in the light of

the new revelations, as discussed here, and need to evolve as more knowledge accumulates.

ACKNOWLEDGMENTS

We dedicate this article to three friends and lambdologists, Hatch Echols, Nat Sternberg, and Ira Herskowitz, who made seminal contributions to science and were devoted to the biology of lambda.

This research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer, and by a Trans NIH/FDA Intramural Biodefense Program Grant from NIAID to D.L.C. The research carried out at Hebrew University was supported in part by The Israel Science Foundation (grants 489/01-1 and 340/04).

Amos B. Oppenheim passed away after submission of the manuscript.

REFERENCES

1. **Atsumi, S., and J. W. Little.** 2004. Regulatory circuit design and evolution using phage lambda. Genes Dev. **18:**2086–2094.
2. **Atsumi, S., and J. W. Little.** 2006. Role of the lytic repressor in prophage induction of phage λ as analyzed by a module-replacement approach. Proc. Natl. Acad. Sci. USA. **103:**4558–4563.
3. **Aurell, E., S. Brown, J. Johanson, and K. Sneppen.** 2002. Stability puzzles in phage lambda. Phys. Rev. E **65:**051914–051919.
4. **Banuett, F., M. A. Hoyt, L. McFarlane, H. Echols, and I. Herskowitz.** 1986. *hflB*, a new *Escherichia coli* locus regulating lysogeny and the level of bacteriophage lambda cII protein. J. Mol. Biol. **187:**213–224.
5. **Britton, R. A., B. S. Powell, S. Dasgupta, Q. Sun, W. Margolin, J. R. Lupski, and D. L. Court.** 1998. Cell cycle arrest in Era GTPase mutants: a potential growth rate-regulated checkpoint in *Escherichia coli*. Mol. Microbiol. **27:**739–750.
6. **Chattopadhyay, S., J. Garcia-Mena, J. DeVito, K. Wolska, and A. Das.** 1995. Bipartite function of a small RNA hairpin in transcription antitermination in bacteriophage λ. Proc. Natl. Acad. Sci. USA. **92:**4061–4065.
7. **Cheng, S. W., D. L. Court, and D. I. Friedman.** 1995. Transcription termination signals in the nin region of bacteriophage λ: identification of Rho-dependent termination regions. Genetics **140:**875–887.
8. **Costantino, N., M. Zuber, and D. Court.** 1990. Analysis of mutations in the *ninR* region of bacteriophage lambda that bypass a requirement for lambda N antitermination. J. Bacteriol. **172:**4610–4615.
9. **Court, D., L. Green, and H. Echols.** 1975. Positive and negative regulation by the cII and cIII gene products of bacteriophage lambda. Virology **63:**484–491.
10. **Court, D. L., T. A. Patterson, T. Baker, N. Costantino, X. Mao, and D. I. Friedman.** 1995. Structural and functional analyses of the transcription-translation proteins NusB and NusE. J. Bacteriol. **177:**2589–2591.
11. **Das, A.** 1993. Control of transcription termination by RNA-binding proteins. Annu. Rev. Biochem. **62:**893–930.
12. **Das, A., B. Ghosh, S. Barik, and K. Wolska.** 1985. Evidence that ribosomal protein S10 itself is a cellular component necessary for transcription antitermination by phage λ N protein. Proc. Natl. Acad. Sci. USA. **82:**4070–4074.
13. **Datta, A. B., S. Roy, and P. Parrack.** 2005. Role of C-terminal residues in oligomerization and stability of λ CII: implications for lysis-lysogeny decision of the phage. J. Mol. Biol. **345:**315–324.
14. **DeVito, J., and A. Das.** 1994. Control of transcription processivity in phage λ: Nus factors strengthen the termination-resistant state of RNA polymerase induced by N antiterminator. Proc. Natl. Acad. Sci. USA. **91:**8660–8664.
15. **Dodd, I. B., A. J. Perkins, D. Tsemitsidis, and J. B. Egan.** 2001. Octamerization of λ CI repressor is needed for effective repression of P(RM) and efficient switching from lysogeny. Genes Dev. **15:**3013–3022.
16. **Dodd, I. B., K. E. Shearwin, and J. B. Egan.** 2005. Revisited gene regulation in bacteriophage lambda. Curr. Opin. Genet. Dev. **15:**145–152.
17. **Dodd, I. B., K. E. Shearwin, A. J. Perkins, T. Burr, A. Hochschild, and J. B. Egan.** 2004. Cooperativity in long-range gene regulation by the λ CI repressor. Genes Dev. **18:**344–354.
18. **Echols, H., L. Green, R. Kudrna, and G. Edlin.** 1975. Regulation of phage λ development with the growth rate of host cells: a homeostatic mechanism. Virology **66:**344–346.
19. **Eisen, H., L. Pereira da Silva, and F. Jacob.** 1968. The regulation and mechanism of DNA synthesis in bacteriophage λ. Cold Spring Harbor Symp. Quant. Biol. **33:**755–764.
20. **Folkmanis, A., W. Maltzman, P. Mellon, A. Skalka, and H. Echols.** 1977. The essential role of the cro gene in lytic development by bacteriophage λ. Virology **81:**352–362.
21. **Friedman, D., and D. Court.** 2006. Regulation of gene expression by transcription termination and antitermination, p. 83–103. *In* R. Calendar (ed.), The bacteriophages, 2nd ed. Oxford University Press, New York, NY.
22. **Friedman, D. I., and D. L. Court.** 2001. Bacteriophage λ: alive and well and still doing its thing. Curr. Opin. Microbiol. **4:**201–207.
23. **Friedman, D. I., and D. L. Court.** 1995. Transcription antitermination: the λ paradigm updated. Mol. Microbiol. **18:**191–200.
24. **Friedman, D. I., and M. Gottesman.** 1983. Lytic mode of λ development, p. 21–51. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor Laboratory, New York, NY.
25. **Galland, P., P. Bassi, and E. Calef.** 1973. On the mode of antirepressor action in anti-immune cells. Mol. Gen. Genet. **125:**231–239.
26. **Guarneros, G.** 1988. Retroregulation of bacteriophage λ *int* gene expression. Curr. Top. Microbiol. Immunol. **136:**1–19.
27. **Guarneros, G., and J. M. Galindo.** 1979. The regulation of integrative recombination by the b2 region and the cII gene of bacteriophage λ. Virology **95:**119–126.
28. **Guarneros, G., C. Montanez, T. Hernandez, and D. Court.** 1982. Posttranscriptional control of bacteriophage λ gene expression from a site distal to the gene. Proc. Natl. Acad. Sci. USA. **79:**238–242.
29. **Herskowitz, I., and D. Hagen.** 1980. The lysis-lysogeny decision of phage λ: explicit programming and responsiveness. Annu. Rev. Genet. **14:**399–445.
30. **Ho, Y. S., and M. Rosenberg.** 1985. Characterization of a third, cII-dependent, coordinately activated promoter on phage λ involved in lysogenic development. J. Biol. Chem. **260:**11838–11844.
31. **Hoopes, B. C., and W. R. McClure.** 1985. A cII-dependent promoter is located within the Q. gene of bacteriophage λ. Proc. Natl. Acad. Sci. USA. **82:**3134–3138.
32. **Hoyt, M. A., D. M. Knight, A. Das, H. I. Miller, and H. Echols.** 1982. Control of phage λ development by stability and synthesis of cII protein: role of the viral cIII and host *hflA*, *himA* and *himD* genes. Cell **31:**565–573.
33. **Johnson, A. D., C. O. Pabo, and R. T. Sauer.** 1980. Bacteriophage λ repressor and cro protein: interactions with operator DNA. Methods Enzymol. **65:**839–856.
34. **Kaiser, A. D.** 1957. Mutations in a temperate bacteriophage affecting its ability to lysogenize *Escherichia coli*. Virology **3:**42–61.
35. **Kobiler, O., S. Koby, D. Teff, D. Court, and A. B. Oppenheim.** 2002. The phage λ CII transcriptional activator carries a C-terminal domain signaling for rapid proteolysis. Proc. Natl. Acad. Sci. USA. **99:**14964–14969.
36. **Kobiler, O., A. Rokney, N. Friedman, D. L. Court, J. Stavans, and A. B. Oppenheim.** 2005. Quantitative kinetic analysis of the bacteriophage λ genetic network. Proc. Natl. Acad. Sci. USA. **102:**5310–5311.
37. **Kourilsky, P., and A. Knapp.** 1974. Lysogenization by bacteriophage λ. III. Multiplicity dependent phenomena occurring upon infection by λ. Biochimie **56:**1517–1523.
38. **Lederberg, E.** 1951. Lysogenicity in *E. coli* K12. Genetics **36:**560–569.
39. **Lozeron, H. A., J. E. Dahlberg, and W. Szybalski.** 1976. Processing of the major leftward mRNA of coliphage λ. Virology **71:**262–277.
40. **Mason, S. W., and J. Greenblatt.** 1991. Assembly of transcription elongation complexes containing the N protein of phage λ and the *Escherichia coli* elongation factors NusA, NusB, NusG, and S10. Genes Dev. **5:**1504–1512.
41. **McAdams, H. H., and L. Shapiro.** 1995. Circuit simulation of genetic networks. Science **269:**650–656.
42. **Miller, H. I., J. Abraham, M. Benedik, A. Campbell, D. Court, H. Echols, R. Fischer, J. M. Galindo, G. Guarneros, T. Hernandez, D. Mascarenhas, C. Montanez, D. Schindler, U. Schmeissner, and L. Sosa.** 1981. Regulation of the integration-excision reaction by bacteriophage λ. Cold Spring Harbor Symp. Quant. Biol. **45:**439–445.
43. **Nodwell, J. R., and J. Greenblatt.** 1993. Recognition of *boxA* antiterminator RNA by the *E. coli* antitermination factors NusB and ribosomal protein S10. Cell **72:**261–268.
44. **Nodwell, J. R., and J. Greenblatt.** 1991. The nut site of bacteriophage λ is made of RNA and is bound by transcription antitermination factors on the surface of RNA polymerase. Genes Dev. **5:**2141–2151.
45. **Oppenheim, A. B., O. Kobiler, J. Stavans, D. L. Court, and S. Adhya.** 2005. Switches in bacteriophage λ development. Annu. Rev. Genet. **102:**4470–4475.
46. **Patterson, T. A., Z. Zhang, T. Baker, L. L. Johnson, D. I. Friedman, and D. L. Court.** 1994. Bacteriophage λ N-dependent transcription antitermination. Competition for an RNA site may regulate antitermination. J. Mol. Biol. **236:**217–228.
47. **Ptashne, M.** 1992. A genetic switch. Blackwell Scientific Publications, Cambridge, United Kingdom.
48. **Ptashne, M.** 2004. Genetic switch: phage lambda revisited, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
49. **Ptashne, M.** 2006. Lambda's switch: lessons from a module swap. Curr. Biol. **16:**R459–R462.
50. **Rees, W. A., S. E. Weitzel, T. D. Yager, A. Das, and P. H. von Hippel.** 1996. Bacteriophage λ N protein alone can induce transcription antitermination in vitro. Proc. Natl. Acad. Sci. USA. **93:**342–346.
51. **Reichardt, L. F.** 1975. Control of bacteriophage lambda repressor synthesis after phage infection: the role of the N, cII, cIII and cro products. J. Mol. Biol. **93:**267–288.
52. **Revet, B., B. von Wilcken-Bergmann, H. Bessert, A. Barker, and B. Muller-Hill.** 1999. Four dimers of lambda repressor bound to two suitably spaced

pairs of lambda operators form octamers and DNA loops over large distances. Curr. Biol. **9:**151–154.
53. **Roberts, J. W.** 1993. RNA and protein elements of *E. coli* and lambda transcription antitermination complexes. Cell **72:**653–655.
54. **Roberts, J. W.** 1969. Termination factor for RNA synthesis. Nature **224:** 1168–1174.
55. **Roberts, J. W., W. Yarnell, E. Bartlett, J. Guo, M. Marr, D. C. Ko, H. Sun, and C. W. Roberts.** 1998. Antitermination by bacteriophage lambda Q protein. Cold Spring Harbor Symp. Quant. Biol. **63:**319–325.
56. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13:** 319–353.
57. **Rosenberg, M., D. Court, H. Shimatake, C. Brady, and D. L. Wulff.** 1978. The relationship between function and DNA sequence in an intercistronic regulatory region in phage lambda. Nature **272:**414–423.
58. **Schindler, D., and H. Echols.** 1981. Retroregulation of the *int* gene of bacteriophage λ: control of translation completion. Proc. Natl. Acad. Sci. USA. **78:**4475–4479.
59. **Schmeissner, U., D. Court, K. McKenney, and M. Rosenberg.** 1981. Positively activated transcription of lambda integrase gene initiates with UTP in vivo. Nature **292:**173–175.
60. **Schmeissner, U., D. Court, H. Shimatake, and M. Rosenberg.** 1980. Promoter for the establishment of repressor synthesis in bacteriophage λ. Proc. Natl. Acad. Sci. USA. **77:**3191–3195.
61. **Squires, C. L., and D. Zaporojets.** 2000. Proteins shared by the transcription and translation machines. Annu. Rev. Microbiol. **54:**775–798.
62. **Steege, D. A., K. C. Cone, C. Queen, and M. Rosenberg.** 1987. Bacteriophage lambda N gene leader RNA. RNA processing and translational initiation signals. J. Biol. Chem. **262:**17651–17658.
63. **Sternberg, N., and L. Enquist.** 1979. Analysis of coliphage lambda mutations that affect Q gene activity: *puq*, *byp*, and *nin5*. J. Virol. **30:**1–13.
64. **Svenningsen, S. L., N. Costantino, D. L. Court, and S. Adhya.** 2005. On the role of Cro in lambda prophage induction. Proc. Natl. Acad. Sci. USA. **102:**4465–4469.
65. **Tomoyasu, T., K. Yamanaka, K. Murata, T. Suzaki, P. Bouloc, A. Kato, H. Niki, S. Hiraga, and T. Ogura.** 1993. Topology and subcellular localization of FtsH protein in *Escherichia coli*. J. Bacteriol. **175:**1352–1357.
66. **Whalen, W. A., and A. Das.** 1990. Action of an RNA site at a distance: role of the nut genetic signal in transcription antitermination by phage-lambda N gene product. New Biol. **2:**975–991.
67. **Wilson, H. R., D. Yu, H. K. Peters, 3rd, J. G. Zhou, and D. L. Court.** 2002. The global regulator RNase III modulates translation repression by the transcription elongation factor N. EMBO J. **21:**4154–4161.
68. **Wilson, H. R., J. G. Zhou, D. Yu, and D. L. Court.** 2004. Translation repression by an RNA polymerase elongation complex. Mol. Microbiol. **53:**821–828.
69. **Zhu, X. M., L. Yin, L. Hood, and P. Ao.** 2004. Calculating biological behaviors of epigenetic states in the phage lambda life cycle. Funct. Integr. Genomics **4:**188–195.